Reviewer’s report

Title: Microarray Analysis Reveals Genetic Pathways Modulated by Tipifarnib in Acute Myeloid Leukemia

Version: 1 Date: 4 August 2004

Reviewer: Jason Gotlib

Reviewer’s report:

General

The manuscript by Raponi et al is the first to describe the application of microarray analysis technology to uncover potential targets of farnesyltransferase inhibitor (FTI) therapy. FTIs have shown efficacy in both chronic and acute myeloid malignancies. In particular, the FTI tipifarnib has elicited remission rates in the range of 20-30% in patients with refractory/relapsed acute myeloid leukemia (AML), and in poor-risk/elderly AML patients previously untreated with chemotherapy. Although several studies have firmly established that FTIs can inhibit the enzyme farnesyltransferase, thus far it has been a “black box” with regard to which cellular targets/pathways are operative in the action of FTIs. Identification of these targets will potentially help stratify which patients are likely to be responders, and should be useful in establishing biologic markers of response and resistance.

Three AML cell lines and cells from 2 AML patients treated with tipifarnib were analyzed for this manuscript. Although patient numbers are low, and more patient samples are clearly needed to generate clinically useful data regarding the action of FTIs such as tipifarnib, this research does indicate the potential usefulness of this approach. I believe the manuscript merits publication, but pending that the authors address the questions and revisions listed.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. P6-7. The text states that “Seventy-two of these genes were affected in patient samples (p<.05, FDR <.03) and were, therefore considered to be significantly regulated by tipifarnib.” Later, on page 8, it is indicated that 1016 genes were significantly changed during farnesyl transferase inhibition in vivo. Please address this inconsistency.

2. Page 8: Broadly speaking, what proportion of the 1198 genes cited to be significantly regulated in at least one of the cell lines were actually significantly regulated in 2 or 3 of the cell lines instead of just one of the cell lines? If a gene was upregulated (or downregulated) in one cell line, was it consistently upregulated (or downregulated) in the other cell lines?

3. Similarly, for the 1016 genes found to be significantly changed in the leukemia cells from patients treated with tipifarnib, was there a persistent upregulation (or downregulation from days 8 to 22) for these genes, or was there substantial variability in genes being upregulated to downregulated (or vice versa) from time point to time point?

4. K-ras is not among one of the genes evaluated by RT-PCR. Given that it is the only one that is significantly regulated (down-regulated) on the microarray, RT-PCR data should be provided for this gene.
5. Was the gene for geranylgeranyltransferase on the microarray, or analyzed by RT-PCR? It would be interesting to evaluate whether this is transcriptionally upregulated with FTI treatment.

6. P9. I believe the reader requires more information about how the Ingenuity Pathway Analysis Tool creates the networks. For example, what defines the limit about the number of genes or the number of associated pathways that will comprise one network? I raise this point because there are 5 associated pathways in network 1 (which also gives the greatest score (24), whereas there are only 3 associated pathways in networks 2-5.

7. Are the 5 networks shown in Table 3 already generated by the Ingenuity Pathway Tool, or are the networks “built” by the authors by entering into the software associated pathways of interest? For example, for network 1, did the authors have to enter “Immunity, inflammation, apoptosis, cell death, and adhesion,” or is this a network pre-defined by the program? If it is the latter, then it would give more reassurance to the reader that the genes found to be significantly regulated on the microarray fell more nicely into pre-established pathways generated by the computer.

8. P. 11, Under “Investigation of apoptosis,” line 8, it is stated that there was a maximum of 23% apoptotic cells at day 5. In looking at the DMSO control, there is approximately 8% apoptosis of cells at day 5. Are these differences in apoptosis (8% vs. 23%) statistically significant? For Figure 6B, are the differences in Annexin V and PI staining (37% vs. 14%) statistically significant?

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Avoid the use of patient initials RH and BS throughout the text, tables and figures. Instead use “patient 1” and “patient 2”

2. P 3. Background, 3rd paragraph: It states that RhoE, a second farnesylated small GTPase, is constitutively activated… What specific disease context is this referring to?

3. P. 10, line 4. Please clarify your use of the term “clustered distally”

4. P. 11, Under “Investigation of apoptosis,” line 8, change Fig. 4 to Fig 6.

5. P. 10, line 13: Change “affects” to “effects.”

6. For reference 7, if what is shown is an abstract, consider using instead the updated publication from Blood, May 1, 2004.

7. Reference 9 is the abstract which pre-dated the Blood publication listed in reference number 8. I recommend deleting reference 9 unless it contains information not provided in reference 8.

8. Table 1: Please note if the AML cases were originally de novo or secondary (arising from prior MDS).

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Discretionary Revisions (which the author can choose to ignore)

1. P2. Abstract, line 3: Remove comma between FTI and tipifarnib, and consider putting after tipifarnib (“ZARNESTRATM, FTI R115777”)

3. P3. Background, line 1: Change “investigative therapeutic compound” to “investigative agent”

4. P3. Background, line 1: Delete “leading”

5. P3. Background, line 4: Change ”is a required process” to “is required for”

6. P3. Background, line 4: Delete “therefore”

7. P3. Background, 2nd paragraph, first sentence: Consider change to, “In hematologic cancers, tipifarnib has shown significant inhibition of the …”

8. P3. Background, 2nd paragraph, line 5: Consider change to,” …response rate in patients with refractory or relapsed…

9. P3. Background, 3rd paragraph, line 1: Change “studies on tipifarnib” to “studies with tipifarnib”

10. P4. Background, 2nd to last, and last sentence of first paragraph. Consider change to: “The regulation of these effectors can lead to the modulation of signaling pathways involving cell growth and proliferation, and apoptosis. Thus, FTIs may have complex inhibitory effects on a number of cellular events.”

11. P4. Background, 2nd paragraph, line 8: Consider change to “…clinical evaluation of the compound’s safety and efficacy in humans.”

12. P4. Background, 2nd paragraph, line 11: Delete “with the FTI” before “tipifarnib.”

13. P. 9. 2nd paragraph, line 10: Change to”…post-translationally; however, it …”

P9. In the last sentence of the first paragraph under “Identification of genetic networks affected by tipifarnib,” clarify to state, “The study by Kamasani et al also found cell cycle pathways were repressed and immunity and cell adhesion pathways were activated by FTI treatment.”

14. P. 12, line 2: Delete “the” in “promise in the hematological malignancies (3-9)”

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests: None